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Analysis of 1-O-Alk-1-enylglycerophospholipids of Albacore Tuna (*Thunnus alalunga*) and Their Alterations during Thermal Processing

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The qualitative and quantitative compositions of 1-O-alk-1-enylglycerolipids of albacore tuna (*Thunnus alalunga*) were studied along the canning process. Ether-linked lipids were found as 1-O-alk-1-enyl-2-acylglycerophospholipids (plasmalogens). They were studied by means of their dimethyl acetal (DMA) derivatives resulting from the transmethylation of lipid samples. DMA and fatty acid methyl esters could be simultaneously analyzed by gas chromatography using a polar fused silica capillary column (SP-2330). Three major 1-O-alk-1-enyl ether chains (16:0″, 18:0″, and 18:1″) were identified in albacore lipids using their mass spectra. Phosphatidylethanolamine showed the highest level in plasmalogen content (54.73%). The acyl composition of plasmalogens and diacylphospholipids was studied. During canning, the plasmalogen content showed a great decrease along the different steps of processing (raw, cooked, and sterilized). However, polyunsaturated fatty acids (PUFAs) from the polar fraction did not show any difference. These results suggest that alkenyl ethers are more susceptible to heat damage than PUFA and should be taken into account when damage in the lipid fraction during thermal processing is considered.

INTRODUCTION

For several marine organisms, plasmalogens (1-O-alk-1-enyl-2-acylglycerophospholipids) can be found as important constituents of lipids. They have been found as ubiquitous constituents of many animal cell membranes, and their high content in some animal tissues (Klenk, 1957; Ansell and Hawthorne, 1964) suggests that they play an important role in the structure (Horrocks and Sharma, 1982) and function (Horrocks and Snyder, 1972) of biological membranes.

During the past years, most plasmalogen studies have been carried out to elucidate its content and composition (Kostetskii and Sergeyuk, 1986; Chapelle et al., 1987; Ohshima et al., 1989; Fogerty et al., 1991) in biological matter; however, little information about changes in food products as a result of industrial processing is available (Marmer et al., 1986; Jeong et al., 1991).

Many studies have been done about the important role that alterations of lipids during treatment or storage of foods have on the quality of the final product (Pearson et al., 1977; Gardner, 1979). It is well-known that lipid damage in foods during storage or processing is focused on the reactivity of polyunsaturated fatty acids (PUFAs) (Chan, 1987; Hsieh and Kinsella, 1989); PUFAs are especially abundant in marine lipids (Ackman, 1989) and have received great attention because of their potential role played against certain diseases (Carroll and Braden, 1986). However, the alterations of food plasmalogens in heat processing or storage have been hardly considered until now.

In the present work, qualitative and quantitative analyses of plasmalogens of albacore tuna (*Thunnus alalunga*) lipids have been carried out. Its content has been studied in the most representative phospholipid classes; the acyl composition of plasmalogens has been compared to the acyl composition of diacylphospholipids. Finally, special stress has been given to changes that plasmalogens may undergo as a result of thermal treatment (cooking and sterilization) during canning.
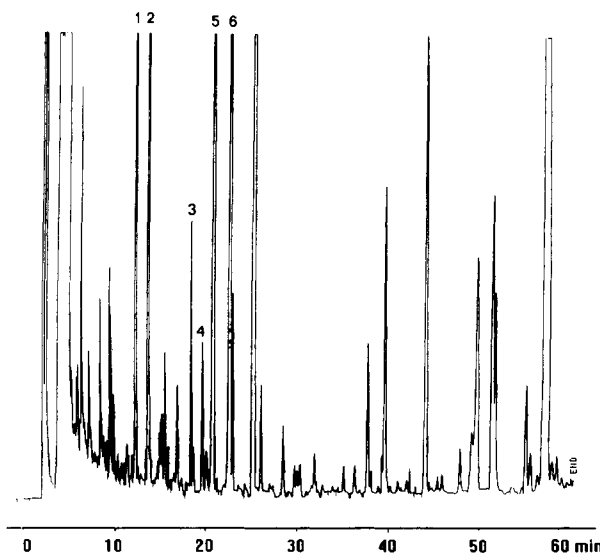
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Figure 1. Gas chromatographic separation of FAME and DMA in albacore muscle. Peaks: 1, 16:0″;2, 16:0; 3, 18:0″; 4, 18:1ω9″; 5, 18:0; 6, 18:1ω9.

MATERIALS AND METHODS

Raw Material, Processing, and Lipid Extraction. Albacore tuna (*T. alalunga*) caught by a tuna fishing vessel on the Atlantic Ocean (approximately 43° N and 27° W) was used. The fish were kept on ice for 3 days. Upon arrival to our laboratory, the whole fish were frozen at −40 °C and stored at −20°C for 1 month prior to analysis. Six individual fish were used (see Table I).

The fish were beheaded and eviscerated. Steam cooking (102–103°C) was performed in our pilot plant to a final backbone temperature of 65°C (around 90 min); then the fish were cooled at room temperature (14 °C) for about 5 h. Fish were skinned, and the red muscle was removed. Each of the six individual fish was considered separately. From raw and cooked white muscle, lipids were directly extracted according to the Bligh and Dyer (1959) method.

Portions of 80–90 g of cooked white muscle were placed in RO-100 cylindrical cans (6.52-cm diameter, 3-cm height). Two kinds of canning were employed: oil canned (addition of 20 mL of olive oil and 2 g of NaCl) and brine canned (addition of 20 mL of aqueous 2% NaCl). The cans were sealed and sterilized in a

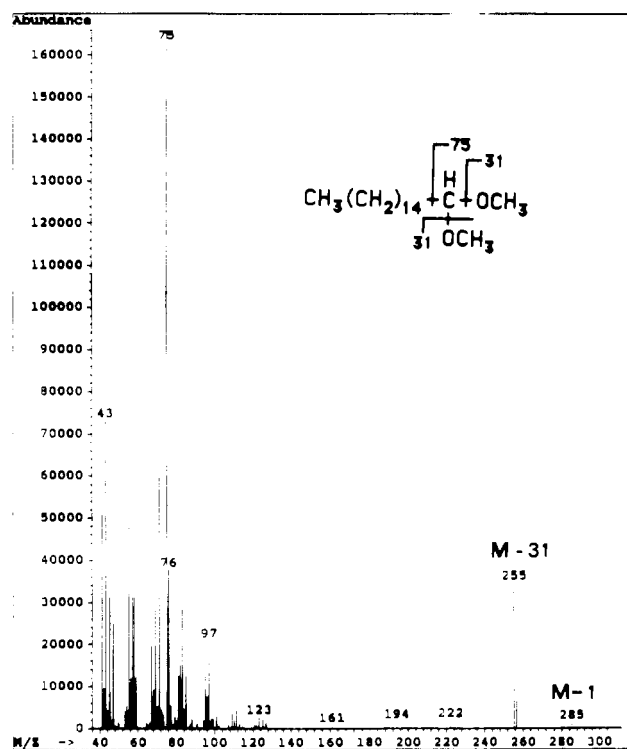2396 J. Agric. Food Chem., Vol. 41, No. 12, 1993

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Figure 2. Mass spectrum of DMA corresponding to acid methanolysis of 1-O-hexadecenoyl- 2-acylglycerophospholipid.

retort under 115°C during 60 min (around F_0 = 7 min). After 3 months of storage at room temperature, the cans were opened and the liquid was drained off carefully; the muscle part was minced and wrapped up with filter paper. Lipids were extracted from the fish flesh following the Bligh and Dyer (1959) method. All organic solvents employed were of reagent grade (E. Merck).

Determination and Purification of Phospholipids. Organic phosphorus was determined on total lipid extracts according to the Raheja et al. (1973) method based in a complex formation with ammonium molybdate (E. Merck). Lipid extracts were subjected to thin-layer chromatography (TLC) on 20 × 20 cm plates of silica gel 60W (0.8 mm) (E. Merck) and developed two times in the same direction with $CHCl_3$–CH_3OH–CH_3COOH (100: 15:2 v/v/v). The polar fraction remained at the bottom and was recovered from silica gel by elution with $CHCl_3$–CH_3OH (2:1 v/v).

Fractionation of Phospholipid Classes. Total phospholipid extracts were resolved by TLC on 20 × 20 cm plates of silica gel 60W (0.25 mm) and developed two times in the same direction in a solvent system of $CHCl_3$–CH_3OH–$CH_3CHOHCH_3$–KCl (0.25%)–$CH_3COOC_2H_5$ (30:9:25:6:18). Individual phospholipids (sum of plasmalogen and diacyl forms) were identified by comparison with phospholipid standards (Matreya, Inc.) and then recovered from silica gel by elution with $CHCl_3$–CH_3OH (2:1) for subsequent gas chromatographic (GC) analysis. From these data, the fatty acid composition of each phospholipid class was established. KCl and all organic solvents were of reactive grade (E. Merck).

To obtain the fatty acid composition of diacyl and plasmalogen forms of individual phospholipids, a combination of high-performance liquid chromatography (HPLC) and GC techniques was carried out. HPLC was performed with a Perkin-Elmer solvent pump, equipped with a silica 60 column (4.6 mm i.d × 25 cm., Supelco, Inc., Bellefonte, PA), a Perkin-Elmer LC-65 UV variable-wavelength detector set at 205 nm, and a Hewlett-Packard 8380A integrator. The separation was achieved with an isocratic elution in a solvent mixture of CH_3CN–CH_3OH–H_3PO_4 (98:1:1). The flow rate was maintained at 1 mL/min. All solvents employed were of HPLC grade (E. Merck).

Polar lipids (150 μg) were injected into the HPLC system, and the phospholipid classes were separated under the conditions described above. Identity of peaks was verified using phospholipid standards. Diacyl forms of phosphatidylinositol (PI),

Table I. Weight and Different Lipid Contents in Six Individual Albacore Tunas

sample	wt[a]	lipid content[b]	phospholipid content[c]	plasmalogen content[d]
1	10.86	16.78 ± 0.07	3.83 ± 0.10	23.58 ± 0.66
2	6.63	7.90 ± 0.02	2.71 ± 0.06	20.82 ± 0.89
3	10.40	4.75 ± 0.01	3.26 ± 0.01	22.36 ± 0.94
4	7.48	6.92 ± 0.01	2.52 ± 0.06	25.00 ± 0.26
5	8.78	16.50 ± 0.09	2.24 ± 0.04	24.07 ± 0.43
6	8.10	9.28 ± 0.08	2.72 ± 0.11	20.22 ± 0.66

[a] Data are expressed in kg. [b] Data are expressed as g of lipids/100 g of dry muscle. [c] Data are expressed as mg of phospholipids/g of dry muscle. [d] Data are expressed as percentage of plasmalogens with respect to polar lipids. Data a–c are the average of three determinations ± standard deviation.
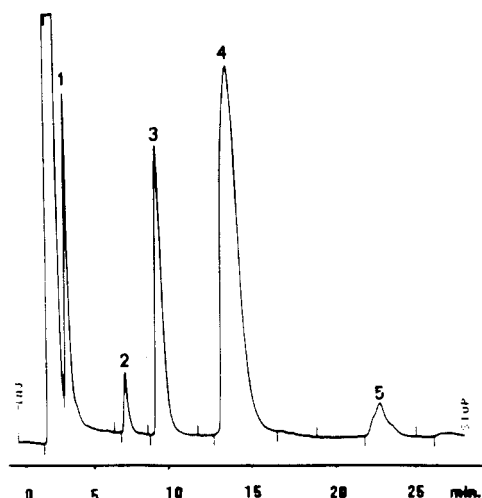

Figure 3. HPLC separation of main phospholipid classes in albacore muscle. Peaks: 1, PI; 2, PS; 3, PE; 4, PC; 5, LPC.

phosphatidylserine (PS), phosphatidylethanolamine (PE), phosphatidylcholine (PC), and lysophosphatidylcholine (LPC) were separated completely, whereas plasmalogens and other minor polar classes eluted with the eluent front. As a result of this separation, purified diacyl forms corresponding to PE and PC classes were obtained and were transmethylated to obtain their acyl composition. Fatty acid composition of PE and PC plasmalogens was then calculated by difference between the results obtained for GC analysis from TLC (composition of sum of plasmalogen and diacyl forms) and GC analysis from purified diacyl forms obtained by HPLC for each phospholipid. To eliminate the excess of H_3PO_4 prior to the transmethylation, a washing step with an alkaline solution (6% K_2CO_3) (E. Merck) was necessary.

RESULTS

In the chromatograms of FAME from polar lipid extracts of albacore tuna, three peaks not assigned in previous works on albacore lipids (Gallardo et al., 1989; Medina et al., 1992) were detected (Figure 1). Identification of these three compounds was achieved by mean of their mass spectra (Figure 2). All of them showed a characteristic peak of 75 mass units, corresponding to the ion ^+CH-$(OCH_3)_2$. This fragment allowed the identification of them as compounds resulting from acidic methanolysis of 1-O-alk-1-enylglycerolipids. The presence of these compounds has already been demonstrated in several marine invertebrates and fishes (Sargent, 1989).

A fragment of 75 m/z does not correspond to a typical FAME mass spectrum, and it allowed a clear differentiation between the two kinds of compounds (DMA and FAME) in the gas chromatogram of transmethylated albacore lipids.

No overlappings were found in the chromatographic signals after different points in peaks (start, middle, and

Table II. Plasmalogen Content and 1-O-Alk-1-enyl Ether Composition of Major Phospholipids in Six Tuna Samples[a]

	16.0″	18.0″	18.1″	total
PE	29.78 ± 0.03	15.51 ± 0.70	9.44 ± 0.31	54.73 ± 2.47
PC	21.73 ± 0.67	2.98 ± 0.47	1.76 ± 0.53	26.67 ± 1.72

[a] Data are expressed as percentage of each plasmalogen in the phospholipid class (mean ± standard deviation).

Table III. Fatty Acid Composition (Percent) of Acyl Chain of Alkenylacyl and Diacyl PE and PC[a]

	1-O-alkenyl-acyl PE	1-O-alkenyl-acyl PC	diacyl PE	diacyl PC
14.0	1.78 ± 0.30	0.00 ± 0.00	3.62 ± 0.22	0.93 ± 1.01
15.0	1.08 ± 0.23	0.00 ± 0.00	1.48 ± 0.29	1.33 ± 1.08
16.0	11.58 ± 1.20	17.09 ± 0.15	9.78 ± 1.20	17.58 ± 1.85
16.1ω7	tr	1.31 ± 0.16	tr	0.53 ± 0.26
16.1ω7	0.62 ± 0.02	3.21 ± 0.44	2.22 ± 0.11	1.16 ± 0.24
16.1ω5	0.63 ± 0.05	0.00 ± 0.25	0.96 ± 0.15	0.56 ± 0.25
17.0	2.30 ± 0.07	1.55 ± 0.15	1.30 ± 0.20	0.97 ± 0.15
18.0	18.67 ± 1.23	3.44 ± 1.73	19.83 ± 1.28	6.20 ± 1.73
18.1ω9	8.94 ± 0.57	12.89 ± 2.25	8.38 ± 0.53	11.86 ± 2.24
18.1ω7	7.03 ± 0.38	0.00 ± 0.38	1.39 ± 0.27	1.41 ± 0.38
18.2ω6	0.46 ± 0.08	2.69 ± 0.15	1.12 ± 0.23	1.41 ± 0.14
18.3ω3	0.97 ± 0.25	0.00 ± 0.06	1.19 ± 0.08	0.75 ± 0.06
20.1ω9	0.93 ± 0.06	0.00 ± 0.00	0.97 ± 0.11	1.30 ± 0.57
20.4ω6	1.70 ± 0.13	5.61 ± 0.34	2.65 ± 0.54	2.45 ± 0.62
20.4ω3	0.58 ± 0.04	0.00 ± 0.00	0.54 ± 0.10	0.57 ± 0.16
20.5ω3	0.51 ± 0.01	5.33 ± 0.66	9.02 ± 0.93	4.12 ± 0.63
24.0	1.64 ± 0.64	tr	1.07 ± 0.10	1.33 ± 0.55
24.1ω9	1.33 ± 0.02	tr	1.73 ± 0.30	2.14 ± 1.10
22.4ω6	0.43 ± 0.04	0.71 ± 0.10	2.21 ± 0.51	1.11 ± 0.10
22.5ω3	3.08 ± 0.16	0.67 ± 0.20	0.59 ± 0.04	1.43 ± 0.50
22.6ω3	36.22 ± 3.32	45.19 ± 2.05	29.93 ± 2.38	37.43 ± 4.89
ΣST	38.13 ± 0.94	22.89 ± 0.58	37.88 ± 1.05	30.67 ± 1.89
ΣMU	20.03 ± 0.49	19.18 ± 0.58	16.43 ± 0.88	21.47 ± 1.47
ΣPUFA	41.84 ± 2.03	58.55 ± 0.96	45.69 ± 1.97	47.86 ± 1.30

[a] Data are expressed as mean ± standard deviation for six samples.

end) were checked. In fact, it has been proved that the use of a very polar fused silica capillary column (SP-2330) allowed the simultaneous analysis of FAME and DMA. Previous studies of FAME and DMA compositions have required the use of prior chromatographic separations and chemical and enzymatic derivatizations (Dembitsky, 1988; Knörr and Spiteller, 1990; Jeong et al., 1991).

From their mass spectra, DMA were identified as a result of the methanolysis of alkenyl ether chains corresponding to 16:0, 18:0, and 18:1 fatty acids (16:0″, 18:0″ and 18:1″, respectively). This assignation agrees with the retention times relative to FAME. DMA eluted earlier than their corresponding methyl esters (Figure 1) (Myer and Kuksis, 1984).

In our study the percentual composition of alkenyl ether chains obtained for albacore tuna was 69.66 ± 2.65% 16:0″, 19.20 ± 1.42% 18:0″, and 11.14 ± 1.14% 18:1″. The same ether chains were obtained by Oshima et al. (1989), although in lower levels in the case of bonito (*Euthynnus pelamis*). Raw albacore was found to be rich in the three corresponding fatty acids, especially for 16:0 fatty acid (Aubourg et al., 1990) in total lipids.

Other alkenyl ether chains (17:0″ and 21:0″) were identified, although their content was very low (below

0.2%). 1-O-Alkyl-2,3-acylglycerolipids were also checked, but they were not detected in significant levels.

The comparative study of DMA content in total lipids and total phospholipids showed that fatty ethers were only present in the polar fraction as plasmalogens (1-O-alk-1-enylphospholipids). A similar finding was obtained for several fishes and marine organisms (Chapelle et al., 1987).

Table I shows the content in total lipids, phospholipids, and plasmalogens for six different albacore tunas. As it can be seen, although lipid content is rather variable, phospholipid and plasmalogen levels seem to be independent of it, and in spite of the different sizes of the individuals, they are not subjected to large content variations. According to Kostetskii and Sergeyuk (1986), the plasmalogen and phospholipid contents did not show any significant variations between individuals caught in the same season.

Composition in DMA and FAME obtained after transmethylation of the different phospholipid classes isolated by TLC showed that only PE and PC (predominant classes in polar fraction: 45% and 20%, respectively) were found as plasmalogens and diacyl forms. Other phospholipids such as PI and PS were present only as diacyl forms. Figure 3 shows the HPLC separation of glycerophospholipids of albacore muscle. The purity of the collected fractions was tested by TLC, and none of the fractions showed DMA peaks in GC.

Table II shows the content of the three kinds of alkenyl ether chains in PE and PC. PE showed the largest content in plasmalogens. A high proportion of 18:0″ was found in PE, corresponding with the high content of 18:0 fatty acid that was found in the acyl positions in both PE plasmalogens and PE diacylphospholipids (Table III). PC showed higher 16.0″ than 18:0″ content. These results are in agreement with those found by Chapelle et al. (1987) and Roots and Johnston (1968) for different marine organisms.

Analysis of the acyl composition of plasmalogens in PE and PC was made. Data obtained for plasmalogen and diacyl compounds were compared. Saturated (ST) fatty acids showed higher levels in PE derivatives than their corresponding PC homologues. In the case of PC, total PUFA showed a larger content in plasmalogen molecules than in the diacyl ones; this result agrees with the general rule of preferential location of PUFA in the 2-position (Litchfield, 1972) but was not clearly obtained for PE.

Thermal Processing. The effect of heat during canning processing (cooking and sterilization) on alkenyl ethers and fatty acids from phospholipids was studied. Results obtained from the six samples (Table IV) were studied by means of an ANOVA. In a previous paper, fatty acids of total lipids were studied in three different zones of albacore tuna muscle along the different steps of the process (Aubourg et al., 1990, 1991). No significant differences were obtained in ST, monounsaturated (MU), and PUFA compositions as a result of thermal treatment. In this study, we found that the acyl composition did not show significant differences among the three steps of processing. No differences were found between oil and

Table IV. Changes in Alkenyl Chain Compositions (Percent) of the Alkenylacyl Subclasses during Thermal Processing[a]

	16.0	18.0	18.1	SAT	MU	PUFA
raw	7.27 ± 0.47[a]	2.01 ± 0.21[a]	1.17 ± 0.14[a]	25.50 ± 1.19	17.50 ± 0.49	46.89 ± 1.44
cooked	8.99 ± 0.67[a]	2.45 ± 0.13[a]	1.37 ± 0.15[a]	30.04 ± 2.65	18.02 ± 0.75	44.36 ± 1.14
brine canned	3.86 ± 0.29[b]	1.09 ± 0.08[b]	0.61 ± 0.05[b]	30.15 ± 0.84	17.99 ± 0.30	48.62 ± 0.92
oil canned	4.07 ± 0.37[b]	1.01 ± 0.16[b]	0.63 ± 0.09[b]	33.41 ± 0.82	18.44 ± 0.43	45.50 ± 1.01

[a] Data are expressed as mean ± standard deviation for six samples. Values in the same column with different superscripts (a and b) are significantlly different (P < 0.05).

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brine canning. However, a second ANOVA performed on the plasmalogen content grouped the samples into two different clusters; one of them was formed by the raw and cooked material (higher levels), and the second one was formed by the two kinds of canned samples (lower levels).

As a result of processing the percentage of PC and PE decreased in the polar fraction (39.02% and 14.55%, respectively).

These results suggest that plasmalogens are more susceptible to damage by heat processing (oxidation and hydrolysis) than their corresponding acyl derivatives. This behavior may be explained by the aldehydogenic chemical structure which is easily open to acidic attacks.

Other studies have demostrated that low temperatures have a different effect on the phospholipid fraction. Experiences carried out at −20°C during 12 months showed that diacyl PC and PE were lost more quickly than the alkenylacyl PC and PE (Jeong et al., 1991). However, during the first 3 months of storage, no significant differences were obtained in the plasmalogen content. A different damage mechanism is supposed to be involved in both kinds of conditions; at frozen temperatures, enzymatic hydrolysis should be predominant (Oshima et al., 1985), while at high temperatures chemical breakdown of weak linkages should be considered as the main effect (Chan, 1987).

Damage to PUFA during thermal processing has been widely studied until now because of the important influence that their oxidation products may have over other kinds of biological molecules in food products (Pearson et al., 1977). In a previous work, Marmer et al. (1986) have demostrated that oxidized PUFA esters accelerate autoxidation of alk-1-enyl ethers. As the thermal treatment does not seem to affect the PUFA esters but clearly alters the ethers, it would be interesting to assess the effect of heat on plasmalogens of oxidized raw material.

Food products derived from biological material possessing high contents of plasmalogens may undergo additional changes as a result of processing that should be also taken into account when quality of human foods is considered. Thus, further studies to elucidate the chemical structure of compounds formed from plasmalogens during high-temperature conditions are recommended.

ABBREVIATIONS USED

DMA, dimethyl acetal; GC, gas chromatography; HPLC, high-performance liquid chromatography; LPC, lysophosphatidylcholine; MU, monounsaturated fatty acid; PC, phosphatidylcholine; PE, phosphatidylethanolamine; PI, phosphatidylinositol; PS, phosphatidylserine; PUFA, polyunsaturated fatty acid; ST, saturated fatty acid; TLC, thin-layer chromatography.

ACKNOWLEDGMENT

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